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Original Article

LncRNA-SNHG3 is an independent prognostic biomarker of intrahepatic cholangiocarcinoma

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Abstract: Background: Numerous deregulated long non-coding RNAs (lncRNAs) accompany the initiation and progression of carcinogenesis. The present study aimed to explore the prognostic significance of lncRNA-SNHG3 on intrahepatic cholangiocarcinoma (ICC) patients. Methods: LncRNA microarray assays were used to evaluate lncRNA expression profiling in three pairs of ICC tissues and adjacent non-tumorous tissues. RT-qPCR was performed to further validate the accuracy of the microarray results. Results: lncRNA microarray and RT-qPCR assays revealed that SNHG3 expression levels were significantly increased in ICC tissues compared to the adjacent non-tumor tissues. A high SNHG3 expression level was significantly correlated with shorter OS in ICC patients. A multivariate regression analysis discovered that SNHG3 could serve as an independent prognostic factor for predicting the OS of ICC patients. Conclusion: We found SNHG3 to be an independent risk factor for predicting the prognosis of ICC. SNHG3 shows a strong promise in the development of novel therapeutic targets for the treatment of ICC.

Keywords: Intrahepatic cholangiocarcinoma, long non-coding RNA, SNHG3, prognosis

Introduction

Intrahepatic cholangiocarcinoma (ICC), frequently located in the biliary tree with epithelial cell malignant growth, is one of the most fatal and the second most common primary hepatic malignant tumor worldwide [1, 2]. Patients with ICC trend to be diagnosed at an advanced stage, and the five-year survival rate of ICC is only 10% [3]. At present, surgical resection is the most effective therapeutic method to improve the survival rate, while surgical operations are only being performed on a minority of ICC patients who present at an early stage [4]. Therefore, exploring novel and specific prognostic indicators is very meaningful for improving the survival outcome in ICC patients.

Current biomarkers, such as carbohydrate antigen 19-9 (CA19-9), CA125, carcinoembryonic antigen (CEA), cytokeratin 7 (CK7) and CK19 as the classic biomarkers, are commonly used in the management of patients with ICC [5-8]. However, the sensitivity and specificity of these markers have been questioned. With the development of microarray and sequencing technology, only 2% of the genome constitutes protein-coding genes in mammals, so numerous non-coding RNAs seemingly are "junk genes" [9]. In fact, non-coding RNAs, including microRNAs (miRNAs), long non-coding RNAs (lncRNAs), and circular RNAs, have attracted much attention due to their large mass and numerous biological functions, including the regulation of transcription and translation, and their function as competing endogenous RNAs and regulators of carcinogenesis [10, 11]. Recently, non-coding RNAs are thought to be independent prognostic biomarkers in various cancer types [12-14]. These studies suggest that non-coding RNAs' potential as prognostic biomarkers may facilitate the translation of basic research into clinical practice.

lncRNAs are characterized by their length of more than 200 bp and their non-protein encoding transcript and have been validated frequently by their dysregulated expression in a variety of tumors, including CCA [15]. Intriguingly, many lncRNAs have been reported as poten-

tial prognostic factors for many human cancers, such as hepatocellular carcinoma [16], non-small cell lung cancer [17], and breast cancer [18]. To our knowledge to date, several lncRNAs, including CCAT, TUG1, and CRNDE, may serve as potential molecular biomarkers for predicting the prognosis of ICC [4, 19, 20]. In the present study, we examined the lncRNA expression profiles in ICC and adjacent non-tumorous tissues to investigate their potential use as prognostic markers of ICC. We found that lncRNA-SNHG3 was elevated in tumor tissues from ICC patients. More importantly, lncRNA-SNHG3 could function as an independent prognostic biomarker of ICC.

Material and methods

Patients and specimens

A total of fifty-two ICC patients were enrolled in our study from January 2009 to June 2012. Tissue-based specimens were collected from fifty-two ICC patients who had undergone surgery. None of the patients enrolled received any radiotherapy or chemotherapy before their operations. All the tumors from the ICC patients were clinically and histologically diagnosed with ICC. All specimens were immediately preserved in liquid nitrogen for experimental measurements. Signed informed consent forms were obtained from all ICC patients. Our study was approved by the Ethics Committee of the Second Affiliated Hospital of Kunming Medical University (Kunming, China) according to the Helsinki Declaration.

LncRNAs microarray

Differentially expressed lncRNAs in three pairs of ICC tissues and adjacent non-tumorous tissues were analyzed using the Agilent Human lncRNA Array V.2.0 platform (Agilent Technologies, Santa Clara, CA, USA).

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR)

Total RNA was isolated using RNAiso (Takara, Dalian, China). An RT Kit (Toyobo Co. Ltd, Osaka, Japan) and KAPA SYBR Green FAST qPCR Kit (KAPA, Wilmington, MA, US) were used to measure the expression levels of lncRNAs using an Applied Biosystems 7300 Real-Time PCR System (Thermo Fisher Scientific, Inc.) and normalized to the internal control U6. The lncRNA expression levels were calculated using the $2^{-\Delta\Delta Cq}$ method [21].

Statistical analysis

Data were presented as the mean ± the standard error of the mean. The statistical analysis was performed using SPSS software version 19.0 (IBM Corp., Armonk, NY, USA) and GraphPad Prism Version 7.0 (GraphPad Software, Inc., La Jolla, CA, USA). A Student's *t*-test or a Kruskal-Wallis test was used to analyze the two-group differences. Pearson χ^2 tests were used to evaluate differences between the clinical characteristics and lncRNA-SNHG3 expression levels in the ICC tissues. Overall survival (OA) was calculated using the Kaplan-Meier method with the log-rank test applied for comparison. Univariate and multivariate regression analyses were performed to evaluate the correlation between the lncRNA-SNHG3 expression and OS using the Cox proportional hazard model. $P < 0.05$ was considered to indicate a statistically significant difference.

Results

Differentially expressed lncRNAs in human ICC tissues

Differentially expressed lncRNAs in three pairs of human ICC and adjacent non-tumorous tissues were measured using a lncRNA microarray analysis. The results revealed that the 30 mostly significantly dysregulated lncRNAs were picked out, according to the $Log2|FC| \geq 2.0$, $P < 0.001$ and FDR < 0.001. Among these lncRNAs, 13 and 17 lncRNAs were down-regulated and up-regulated, respectively, in human ICC tissues compared with the adjacent non-tumorous tissues (**Figure 1A**). To further validate the accuracy of the microarray results, the top 5 differentially expressed lncRNAs, including PCAT1, SNHG16, SNHG3, LASP1-AS, and LINC-USP16, were analyzed using RT-qPCR assays. PCAT1, SNHG16, SNHG3, LASP1-AS expression levels were significantly increased, but LINC-USP16 expression levels were markedly decreased in fifty-two ICC tissues as compared to adjacent non-tumor tissues (**Figure 1B**). These results were consistent with the microarray assay. We also found the fold change (FC) of SNHG3 at the maximum value in the top 5 differentially expressed lncRNAs us-

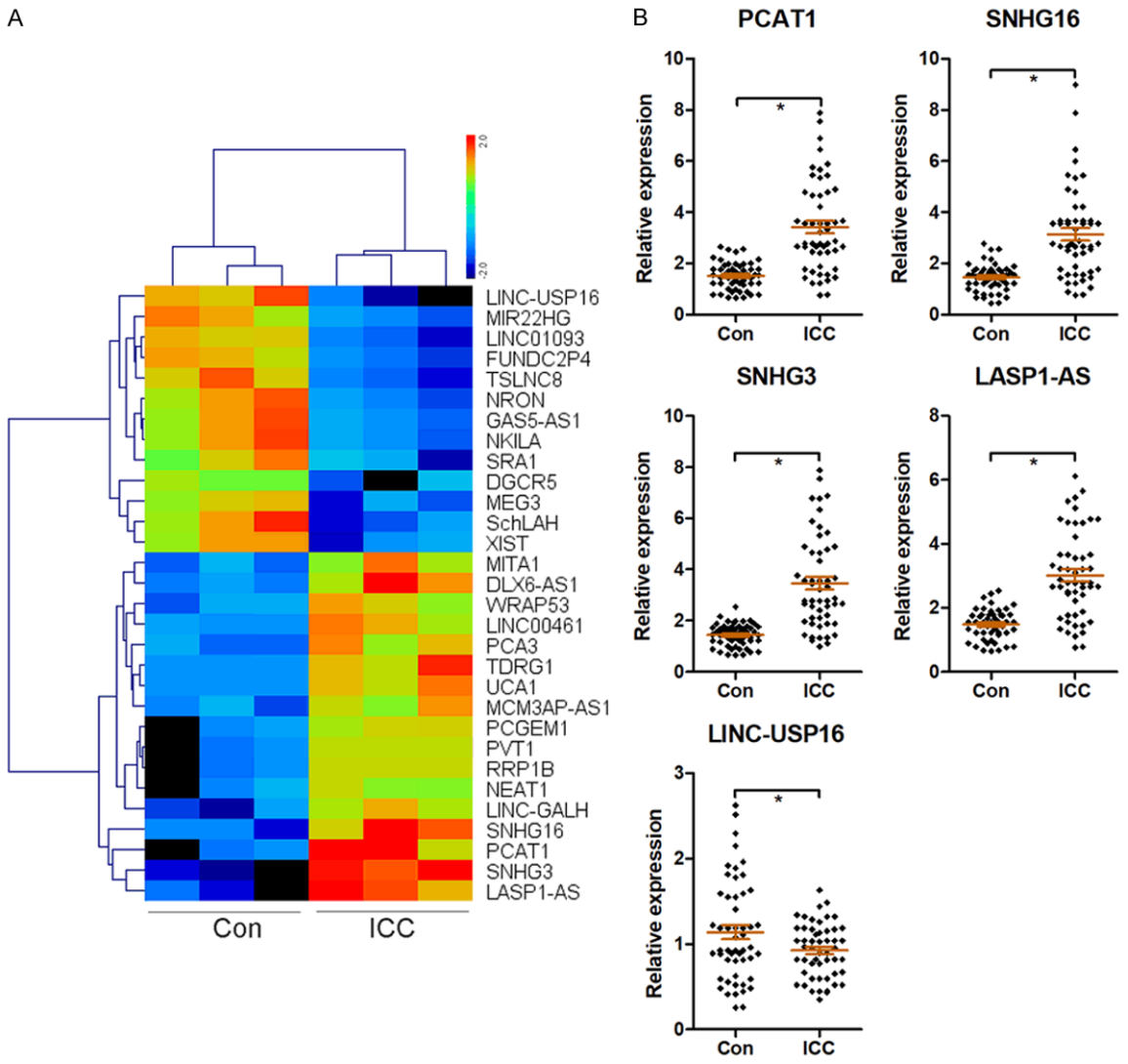

Figure 1. Differentially expressed lncRNAs in human ICC tissues. LncRNA expression profiling in three pairs of human ICC and adjacent non-tumorous tissues was analyzed using an lncRNA microarray, and a heatmap of 30 differentially expressed lncRNAs was presented based on Log2|FC| ≥ 2.0, $P < 0.001$ and FDR < 0.001 (A). The top 5 differentially expressed lncRNAs, including PCAT1, SNHG16, SNHG3, LASP1-AS, and LINC-USP16, were analyzed using RT-qPCR assays (B). *$P < 0.05$.

ing RT-qPCR assays. Therefore, we focused on SNHG3 in the subsequent experiments.

SNHG3 is an independent prognostic biomarker of ICC patients

The relationship between SNHG3 expression and the clinicopathological characteristics of ICC patients is shown in **Table 1**. Pearson χ^2 tests indicated that the expression levels of SNHG3 had a significant correlation with age, gender, serum CA19-9 levels, tumor size, and histological differentiation. However, the up-regulation of the SNHG3 expression level is significantly correlated with poor TNM stage (P = 0.006), lymph node metastasis (P = 0.008), and distant metastasis (P = 0.010) in ICC patients. In addition, the Kaplan-Meier method and the log-rank test showed that age, gender, serum CA19-9 levels, tumor size, and histological differentiation could not be used as indicators for predicting OS of ICC patients (**Figure 2**). Poor TNM stage (P = 0.0086), lymph node metastasis (P = 0.0009), distant metastasis (P = 0.0215) and high SNHG3 expression levels (P = 0.0094) were significantly correlated with short OS in ICC patients (**Figure 2**). Univariate and multivariate regression analyses showed

Table 1. Correlations between the lncRNA-SNHG3 expression levels and the clinicopathological variables of ICC patients

Variables	n	LncRNA-SNHG3 expression		*P* value
		Low (n = 25)	High (n = 27)	
Age (years)				0.554
≤ 60	29	15	14	
> 60	23	10	13	
Gender				0.586
M	27	12	15	
F	25	13	12	
CA19-9 (U/mL)				0.618
≤ 40	19	10	9	
> 40	33	15	18	
Tumor size (cm)				0.376
≤ 3	30	16	14	
> 3	22	9	13	
TNM stage				0.006
I/II	23	16	7	
III/IV	29	9	20	
Histological differentiation				0.262
Well/Moderate	27	15	12	
Poor	25	10	15	
Lymph node metastasis				0.008
No	32	20	12	
Yes	20	5	15	
Distant metastasis				0.010
No	37	22	15	
Yes	15	3	12	

that TNM stage, lymph node metastasis, distant metastasis, and SNHG3 could serve as independent prognostic factors for predicting the OS of ICC patients (**Table 2**).

Discussion

ICC is one of the most infamous malignant tumors in humans and is usually diagnosed at the advanced stages or through distant metastasis, which leads to a poor prognosis [22]. More reliable and effective prognostic markers may provide new ideas for improving the clinical outcomes in ICC patients. Recent studies have demonstrated that numerous lncRNAs are deregulated in ICC [23, 24]. For example, Wang et al. revealed that 2773 lncRNAs and 2392 lncRNAs were significantly up-regulated and down-regulated, respectively, in ICC tissues compared with the noncancerous tissues [23]. Lv et al. performed an lncRNA microarray assays in four pairs of ICC tissues and normal tissues, and found that 2716 lncRNAs are markedly differentially expressed in ICC tissues compared with normal tissues [24]. Moreover, several lncRNAs, such as CCAT1, CRNDE, and CCAT2, promote the initiation and progression of ICC, and the up-regulation of these lncRNAs are associated with poor OS in ICC patients [20, 25, 26].

In our study, SNHG3, as a prominent carcinogenic lncRNA, was significantly increased in ICC tissues compared with adjacent non-tumor tissues, which was validated by a lncRNA microarray and by RT-qPCR assays. Importantly, SNHG3 was identified as an independent prognostic indicator for OS in ICC patients. SNHG3 as an oncogene has been reported in several cancer types, such as osteosarcoma, lung adenocarcinoma, hepatocellular carcinoma, ovarian cancer, and colorectal cancer [27-31]. In two independent cohort studies, patients with high SNHG3 expressions had poorer OS than those with a low expression of SNHG3 in ovarian cancer and osteosarcoma [27, 30]. The TCGA database also shows that high SNHG3 expression is correlated with poor OS in colorectal cancer and lung adenocarcinoma [28, 31]. We also found that ICC patients with high SNHG3 expression exhibited a significant shorter OS than patients with low SNHG3 expression. Moreover, the up-regulation of SNHG3 expression was significantly positively related with poor TNM stage, lymph node metastasis and distant metastasis in ICC patients. Multivariate regression analysis validated that poor TNM stage, lymph node metastasis and distant metastasis could also serve as independent prognostic factors of ICC.

However, there are some limitations to our study. First, the relatively low number of cases may impact the credibility of the conclusions in our study. The functional aspect of lncRNA usually competes with endogenous RNA to sponge miRNA and its downstream target gene [32]. However, we did not investigate the roles of SNHG3 on ICC cells growth *in vitro* and *in vivo*.

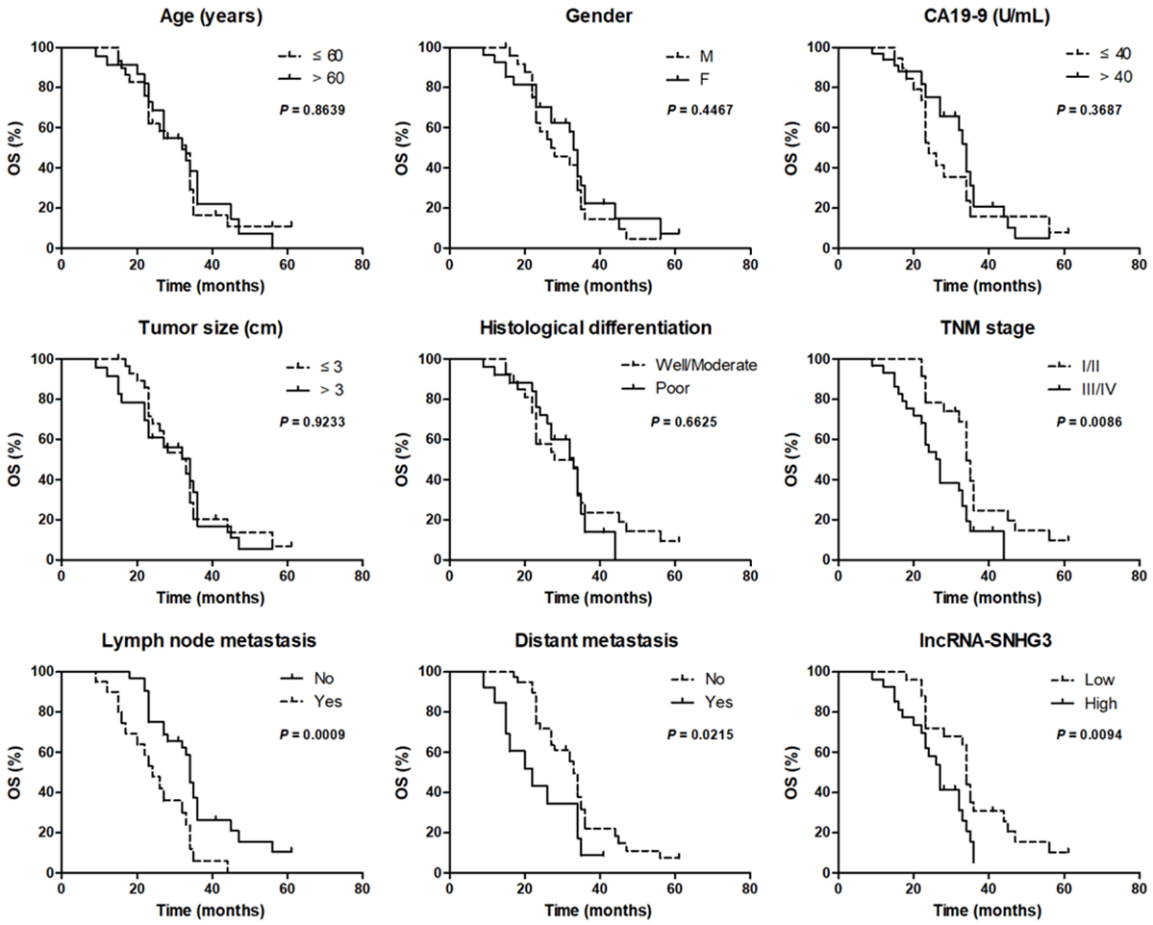

Figure 2. SNHG3 was an independent prognostic biomarker for ICC patients. A Kaplan-Meier survival curve analysis and a log-rank test were applied to evaluate whether age, gender, CA19-19, tumor size, histological differentiation, TNM stage, lymph node metastasis, distant metastasis and lncRNA-SNHG3 expression levels were correlated with the OS of ICC patients.

Table 2. Univariate and multivariate regression analysis of ICC patients for overall survival

Variables	Univariate		Multivariate	
	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
Age (> 60 vs ≤ 60)	1.23 (0.67-2.90)	0.755		
Gender (M vs F)	1.31 (0.75-3.16)	0.562		
CA19-9 (> 40 vs ≤ 40)	1.53 (0.86-3.79)	0.349		
Tumor size (> 3 vs ≤ 3)	1.27 (0.70-3.05)	0.647		
Histological differentiation (Poor vs Well/Moderate)	1.48 (0.81-3.42)	0.455		
TNM stage (III/IV vs I/II)	3.72 (1.84-8.93)	0.003	2.95 (1.42-7.37)	0.011
Lymph nodes metastasis (Yes vs No)	3.44 (1.66-7.94)	0.007	2.48 (1.11-5.73)	0.019
Distant metastasis (Yes vs No)	2.38 (1.04-5.56)	0.022	2.09 (0.96-4.87)	0.041
LncRNA-SNHG3 (high vs low)	3.25 (1.54-7.51)	0.009	2.82 (1.36-7.03)	0.015

In summary, we found SNHG3 to be an independent risk factor for predicting the prognosis of ICC. SNHG3 looks promising as a means of developing a novel therapeutic target for the treatment of ICC.

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Disclosure of conflict of interest

None.

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